Some Magnesium Status Indicators and Oxidative Metabolism Responses to Low-Dietary Magnesium Are Affected by Dietary Copper in Postmenopausal Women

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OBJECTIVE: A study with human volunteers was conducted to ascertain whether a low intake of copper (Cu) would exacerbate the response to a deficient intake of magnesium (Mg).

METHODS: Nineteen postmenopausal women, age 47 to 78 y, completed a metabolic unit study as designed. For 162 d, nine women were fed a diet containing 1.0 mg of Cu/2000 kcal and 10 women were fed 3.0 mg of Cu/2000 kcal. Diets contained 99 or 399 mg of Mg/2000 kcal for 81 d in a randomized, double-blind, crossover design. Differences were considered significant when statistical analysis yielded $P \leq 0.05$.

RESULTS: Magnesium balance was highly positive when the dietary magnesium was high but non-positive when dietary magnesium was low. Copper balance was more positive when dietary copper was high than when it was low. Plasma ionized magnesium was decreased by magnesium deprivation. Several variables measured indicated that low dietary copper affected the response to magnesium deprivation or vice-versa. Red blood cell magnesium was lower when dietary copper was low than when it was high. When dietary magnesium was low, serum copper was lower in the women fed marginal copper than in those fed luxuriant copper. When dietary magnesium was high, low dietary copper did not affect serum copper. Magnesium deprivation decreased red blood cell superoxide dismutase when dietary copper was luxuriant; when dietary copper was low, magnesium deprivation did not have much of an effect. Apolipoprotein A1 was lowest when dietary magnesium and copper were low. The order in which the magnesium restriction occurred affected the response of a number of variables to this treatment including concentrations of serum magnesium and total and low-density lipoprotein cholesterol.

CONCLUSIONS: The findings indicated that, in short-term magnesium depletion experiments, the response to depletion can be influenced by other dietary factors including copper intake and a high magnesium intake before depletion, and that 100 mg of Mg/d is inadequate for postmenopausal women. *Nutrition* 2003;19:617–626. ©Elsevier Inc. 2003

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INTRODUCTION

In animals and humans, deficiencies of copper and magnesium have several similarities. The lack of either element results in undesirable changes in lipid and reactive oxygen species metabolism that lead to diverse pathologies including those in the cardiovascular system and bones. Epidemiologic and experimental studies have suggested that subclinical copper deficiency contributes to an increased risk of coronary heart disease.[1–4] Abnormal electrocardiograms, hyperlipidemia, and blood pressure changes have been observed in humans and animals experimentally depleted of copper. Cardiac lesions and hypertrophy are consistent consequences of severe copper deficiency in animals.[5] Low copper status also has been implicated as a risk factor for osteoporosis in postmenopausal women.[6] Deficient intakes of magnesium also have been suggested as a contributor to the prevalence of cardiovascular disease in Western countries.[7–9] Hypertension, myocardial infarction, cardiac dysrhythmias, coronary vasospasm, and premature atherosclerosis have been linked to chronic magnesium depletion. Epidemiologic evidence has related low magnesium intake to a high incidence of cardiac deaths,[10–12] particularly in soft-water areas where water-borne magnesium is low. Magnesium depletion also has been associated with skeletal disorders as humans age.[9,13]

Because of the similarities, one might expect that the dietary intake of copper would affect the response to magnesium depri-

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TABLE I.

BASAL DIET MENUS*

Meal	Day 1	Day 2	Day 3
Breakfast	Orange Tang†	Cranapple Drink	Orange Tang
	Canadian bacon croissant	Ham cheese omelet	Corn flakes
	Shredded cheddar cheese	English muffin	Nondairy creamer
		Margarine	Sugar
			Croissant
			Strawberry jelly
Lunch	7-Up‡	7-Up	7-Up
	Ham broccoli casserole	Pepperoni pizza	Beef softshell taco
	Cottage cheese, 2%	Lettuce salad	Sour cream
	Cherry crisp	French dressing	Taco sauce
		Sugar cookie	Peach cobbler
Dinner	Lemonade	Lemonade	Lemonade
	Chicken rice casserole	Beef stew	Chicken burger
	Lettuce salad	Cottage cheese, 2%	Hamburger bun
	Ranch dressing	Peach slices	Lettuce leaf
	Sugar cookie	Angel food cake	Mayonnaise
	Vanilla frosting	Vanilla frosting	Tomato soup
			Vanilla wafers
Snack	Cheesecake	Strawberry jello	Lemon pudding
	Mandarin oranges	Strawberries	Cool Whip‡
		Vanilla wafers	

* The diet supplied the following nutrients per 2000 kcal or 8.4 MJ: 74 g protein, 255 g carbohydrate (78 g as fructose), 78 g fat (188 mg as cholesterol), 1865 mg K (596 mg as a potassium gluconate supplement), 966 mg P (54 mg as a dicalcium phosphate supplement), 740 mg Ca (70 mg as a dicalcium phosphate supplement), 26 mg Fe (20 mg as a ferrous gluconate supplement), 99 mg Mg, 13 mg Zn (7 mg as a zinc sulfate supplement), 1 mg Cu (0.6 mg as a copper sulfate supplement), 2.8 mg Mn (2 mg as a manganese sulfate supplement), 1.45 mg B (1 mg as a chelated borate supplement), 1064 retinol equivalents vitamin A, 10.8 μg vitamin D (10 μg from a supplement containing fish liver oils), 5 mg α-tocopherol, 1.5 mg thiamine, 1.5 mg riboflavin, 20 mg niacin, 1.4 mg vitamin B6, 204 mg folate, 3.2 μg vitamin B12, and 191 mg vitamin C.
† Kraft Foods Inc., Rye Brook, NY, USA.
‡ Dr. Pepper/Seven Up, Inc., Plano, TX, USA.

vation and vice-versa. Thus, it is quite surprising that this has apparently received very little research attention. The limited reports on the subject include that of Cadell et al. who found that copper deficiency aggravates acute audiogenic shock (apnea, bradycardia, hypoxia, acidosis, tetany, and unconsciousness) in magnesium-deficient rats.[14] Pellett and Milne found that an interaction between copper and magnesium affects serum cholesterol, ratio of heart weight to body weight, and cytochrome c oxidase activity in rats.[15,16] Enlargement of the ratio of heart weight to body weight in copper-deficient rats was more substantial in magnesium-marginal and magnesium-adequate rats than in magnesium-deficient rats. Increasing dietary magnesium from 100 to 400 μg/g raised serum cholesterol from 84 to 151 mg/dL in copper-adequate rats but reduced serum cholesterol from 189 to 148 mg/dL in copper-deficient rats.[15] Increasing dietary magnesium from 125 to 425 μg/g decreased platelet cytochrome c oxidase in copper-deficient and copper-marginal rats.[16]

The preceding observations suggested a relation between copper and magnesium in which each could affect the metabolism and the need of one mineral for the other. Moreover, because both elements are found in similar foods,[17] the lack of copper or magnesium in the diet most likely indicates the lack of the other. Thus, a combined low intake of copper and magnesium might be a practical concern in human nutrition. The objective of the current study was to determine whether such a concern is tenable.

METHODS AND MATERIALS

Healthy postmenopausal women, age 47 to 78 y, with body mass indexes between 19.3 and 35.7 kg/m^2 were recruited through public advertisements and admitted to the study after they had been informed in detail of the nature of the research and associated risks and after medical, psychological, and nutritional evaluations had established that they had no underlying disease, were not receiving hormone replacement therapy, and were emotionally suited for the study. The participants gave their written informed consent to participate in the experimental protocol, which was approved by the Institutional Review Board of the University of North Dakota and the Human Studies Review Committee of the United States Department of Agriculture and followed the guidelines of the Department of Health and Human Services and the Helsinki Doctrine regarding the use of human subjects.

The women resided in the metabolic unit of the Grand Forks Human Nutrition Research Center under close supervision for 24 h/d, 7 d/wk; when outside the metabolic unit, the women were chaperoned by trained staff. They consumed only food and beverages provided by the dietary staff. The diet was based on ordinary Western foods presented in a 3-d rotating menu cycle (Table I). The energy distribution of the diet was 15% protein, 35% fat, and 50% carbohydrate (16% as fructose). Analysis showed that the basal diet was low in magnesium (99 mg/2000 kcal) and marginally adequate in copper (1 mg/2000 kcal; 0.6 mg as a copper sulfate supplement in breakfast juice). The basal diet provided calcium (740 mg/2000 kcal) and folic acid (204 μg/2000 kcal) in amounts that were close to recommended dietary allowances at the time of the experiment.[18] The diet was adequate in all other nutrients. Dietary iron (26 mg/d) was provided in excess of the recommended dietary allowance to prevent a decline in iron status as a result of phlebotomy during the experiment. All food was weighed with an accuracy of one-tenth of a gram during

preparation in the metabolic kitchen and was eaten quantitatively by the subjects with the aid of spatulas and rinse bottles.

Twenty-eight women were admitted to the study, which was divided into two 172-d phases because the metabolic unit could house only 14 subjects. Nineteen subjects completed the double-blind, randomized, crossover experiment as designed. Upon arrival at the center, the women began a 10-d equilibration period during which the basal diet supplemented with 200 mg (total of 299 mg) of magnesium and 1 mg (total of 2 mg) of copper per day was fed. In each phase of the experiment, the women were assigned to one of two groups ($n = 7$). One group was fed the basal diet containing 1 mg of Cu/2000 kcal, which was below the estimated safe and adequate daily dietary intake established at the time of the experiment.[18] The other group was fed the basal diet supplemented with 2 mg of Cu/d (total of about 3 mg/d), which was the upper limit of the estimated safe and adequate daily dietary intake.[18] Each group participated in two 81-d dietary periods. During one period, the basal diet was not supplemented with magnesium; during the other, it was supplemented with 300 mg of Mg/d as magnesium gluconate in capsules (Willner Chemists, Inc., New York, NY, USA) provided at mealtimes. The dietary intake of each subject was based on energy needs as calculated by the Harris-Benedict equation[19] plus an additional 50% of basal energy expenditure for normal activity. During the study, body weight was maintained within ±2% of admission weight by the use of individualized exercise prescriptions and by adjusting the amount of the basal diet in 200-kcal increments.

For balance determinations, duplicate diets of 2000 kcal were prepared daily for analysis and blended in a plastic blender with stainless steel blades. Urine and feces were collected carefully to avoid mineral element contamination. Venous blood (limited to ≤250 mL/mo) was taken by phlebotomy weekly for routine health assessment; additional blood was taken every 2 wk for the determination of experimental variables.

The magnesium and copper content of 6-d composites of diets and feces were determined by inductively coupled argon plasma emission spectroscopy (Jarrell-Ash, Waltham, MA, USA)[20] after wet digestion of lyophilized blended samples with nitric and perchloric acids.[21] Urinary copper and magnesium were determined by inductively coupled argon plasma emission spectroscopic analysis of a diluted aliquot. Concurrent replicate analysis of a Total Diet SRM 1548 (National Institute of Standards and Technology, Gaithersburg, MD) yielded means ± standard deviations of 2.6 ± 0.1 μg/g for copper and 552 ± 18 μg/g for magnesium compared with certified values (mean ± confidence values) of 2.6 ± 0.3 μg/g for copper and 556 ± 27 μg/g for magnesium. Magnesium and copper balances were calculated as the difference between intake and excretion (feces plus urine) for the last 24 d of each dietary period. The balance or retention of copper and magnesium calculations did not include surface or phlebotomy losses.

After the subjects had fasted overnight for 10 h, blood was drawn into plastic syringes from an antecubital vein that had been distended by the temporary use of a tourniquet. The blood was processed within 90 min to obtain serum or plasma. Ethylene-diamine-tetra-acetic acid was used as the anticoagulant to obtain plasma. Serum magnesium concentrations were determined by flame atomic absorption spectrometry after dilution with a 0.5% solution of lanthanum oxide in deionized water.[22] Plasma copper concentrations were determined by flame atomic absorption spectrometry after dilution with deionized water.[23] Concurrent analysis of 36 Sera Chem controls (Fisher Scientific, Orangeberg, NY) yielded values (mean ± standard deviation) of 16.1 ± 0.8 μM/L for copper and 0.94 ± 0.03 mM/L for magnesium compared with certified values (mean ± confidence values) of 18.9 ± 3.0 μM/L for copper and 0.92 ±l 0.26 mM/L for magnesium. Plasma ionized magnesium was measured by an electrolyte analyzer (Nova 8+, Nova, Waltham, MA). Serum ceruloplasmin (EC 1.16.3.1) was measured enzymatically as *p*-phenylene-diamine oxidase[24] and by radial immunodiffusion (Behring Diagnostics, Somerville, NJ).[25] The results of both assays were normalized to milligrams of ceruloplasmin per liter by using purified human ceruloplasmin (40 U/mg protein; Sigma, St. Louis, MO, USA) as a standard. Serum total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, triacylglycerol, and apolipoprotein (Apo) B were determined by standard methods using the Cobas Fara II Centrifugal Analyzer (Roche Diagnostics Systems, Montclair, NJ, USA). Serum Apo-A1 was determined by the method of Rifai and King, which uses a nephelometer (Behring Nephelometer 100 Analyzer, Behring Diagnostics, Westwood, MA, USA).[26] Platelets, mononucleated white cells, neutrophils, and red blood cells were separated by using a Percoll gradient.[27] After treatment with Picoex S (United Technologies Packard, Downers Grove, IL, USA), a mild detergent, and sonication, cytochrome c oxidase (EC 1.9.3.1) activity in platelets and mononucleated white cells was measured by a method described by Prohaska.[28] Superoxide dismutase (SOD; EC 1.15.1.1) activity in erythrocytes was measured by a method described by Winterbourne et al.[29] Glutathione peroxidase (EC 1.11.1.9) activity[30] in red blood cells and glutathione[31] concentration in whole blood were determined by previously described methods. Red blood cell membranes were obtained according to the method of Dodge et al.[32] Red blood cell and membrane concentrations of copper and magnesium were determined by a published method.[27]

Because potentially harmful electrocardiographic changes have been found in people depleted in copper and magnesium, Holter electrocardiograms were obtained each week in all subjects. Tapes obtained were scanned (Model 363, Accuplus, Del Mar Avionics, Irvine, CA, USA) by skilled nurses. Ventricular premature discharges were identified and counted. If a verified four-fold increase over the baseline value obtained during equilibration occurred in a subject, she was supplemented with copper and/or magnesium until the end of the study.

Changes in some of the biochemical variables appeared only toward the end of each 81-d dietary period. Thus, to ensure that changes were recognized, only the last two measurements of each variable were used in the statistical analysis. The last four 6-d balance periods were statistically analyzed because they encompassed the blood draws for the statistically analyzed biochemical variables. The data were analyzed by three-way repeated measures analysis of variance (dietary copper, magnesium, and magnesium feeding sequence) with a SAS general linear model program (SAS 8.02, SAS Institute, Cary, NC, USA). To determine whether a balance mean was significantly different from zero, the pooled variance estimate in a *t* test was used. $P \leq 0.05$ were considered significant, and $P = 0.05$ and 0.10 were considered approaching significance. Variances in the data are expressed as a pooled standard deviation, calculated as the square root of the mean square error from the analysis of variance. Data from the nine women who did not complete the study as designed were not included in the analysis.

RESULTS

Nineteen women completed the study as designed and are included in the RESULTS. Three women left the study for personal reasons. Three women were dismissed from the study for medical reasons unrelated to the study. Three women on the low copper diet exhibited heart rhythm changes that dictated an increase in dietary copper midway through the study. Overall, however, it was low dietary magnesium that increased ectopy on Holter cardiograms.[33]

Table II shows that magnesium balance was highly positive when the women were consuming about 400 mg of Mg/kcal but was not when the diet provided about 100 mg of Mg/kcal. Urinary magnesium excretion was significantly decreased with dietary magnesium restriction, but the homeostatic response was not enough to prevent non-positive magnesium balance when the

TABLE II.

MAGNESIUM BALANCE (MG/D) DURING THE LAST 24 D OF EACH 81-D DIETARY PERIOD§

		Low dietary Cu (1 mg/2000 kcal)					High dietary Cu (3 mg/2000 kcal)				
Diet Mg	Sequence*	*n*†	Diet Mg	Feces Mg	Urine Mg	Balance	*n*†	Diet Mg	Feces Mg	Urine Mg	Balance
Low	First	4	116‡	63	64	−11	5	122	60	75	−13
High	Second	4	426	208	127	90	5	424	190	146	87
Low	Second	5	120	58	61	1	5	141	60	76	6
High	First	5	390	153	133	104	5	404	173	142	88
Low	Mean	9	118	60	62	−5	10	131	60	75	−3
High	Mean	9	408	181	130	97	10	414	182	144	88

* Sequence in which dietary magnesium was fed in the crossover design; mean is the average of all subjects fed diets low or high in Mg.
† Number of subjects; because each sequence group started with seven subjects, *n* indicates the volunteer dropout distribution.
‡ Values are means. Caloric intake variation resulted in some diet Mg variations.
§ Significant *P* values by repeated measures analysis of variance: feces Mg: Mg = 0.01. Mg × sequence × Cu = 0.005; urine Mg: Mg = 0.0001; Balance: Mg = 0.0001. Pooled standard deviations: 14 for feces Mg, 13 for urine Mg, 23 for balance.
Cu, copper; Mg, magnesium

magnesium restriction was before magnesium supplementation in the crossover design. Dietary copper intake did not significantly affect magnesium balance.

The copper balance data shown in Table III indicate that the women who were fed the diet providing about 3 mg of Cu/d had good copper status because copper retention amounted to a mean of 0.27 mg/d, a value significantly different from zero. The copper status of the women fed the diet providing about 1 mg of Cu/d probably was marginal because copper retention or balance was near zero without considering surface or phlebotomy losses of copper. Dietary magnesium intake did not significantly affect copper balance.

The dietary manipulations did not significantly affect hematocrit or hemoglobin concentration (data not shown). Blood magnesium concentrations were within the range of normal values but did respond to the dietary treatments (Table IV). Ionized magnesium was significantly lower during the magnesium-depletion period than during the period in which dietary magnesium was high. The sequence of the magnesium treatments did not affect this variable. Serum magnesium, however, was affected by the sequence of magnesium intakes. Serum magnesium was lower when dietary magnesium was low than when it was high when the magnesium restriction occurred first. When the magnesium restriction occurred after 81 d of high magnesium intake, the restriction did not affect serum magnesium. Intracellular magnesium also was affected by the dietary treatments. When examined on the hemoglobin basis, the magnesium content of red blood cells was significantly higher when dietary copper was low than when it was high. When expressed on the per red blood cell basis, the effect of dietary copper only approached significance ($P = 0.08$). The magnesium content of packed red blood cells also was significantly higher when dietary copper was low than when it was high. The magnesium concentration in red blood cells expressed as per cell, gram of hemoglobin, or packed cells was lower when dietary

TABLE III.

COPPER BALANCE (MG/D) DURING THE LAST 24 D OF EACH 81-D DIETARY PERIOD*

		Low dietary Cu (1 mg/2000 kcal)					High dietary Cu (3 mg/2000 kcal)				
Diet Mg†	Sequence‡	*n*§	Diet Cu	Feces Cu	Urine Cu	Balance	*n*§	Diet Cu	Feces Cu	Urine Cu	Balance
Low	First	4	1.11‖	0.97	0.04	0.11	5	3.13	2.91	0.05	0.18
High	Second	4	1.08	1.00	0.05	0.03	5	3.09	2.78	0.05	0.27
Low	Second	5	1.02	0.95	0.04	0.03	5	3.05	2.59	0.04	0.41
High	First	5	1.08	0.97	0.04	0.07	5	3.15	2.87	0.04	0.23
Low	Mean	9	1.07	0.96	0.04	0.07	10	3.09	2.75	0.05	0.29
High	Mean	9	1.08	0.99	0.04	0.05	10	3.12	2.82	0.04	0.25

* Significant *P* values by repeated measures analysis of variance: feces Cu: Cu = 0.0001, Mg × sequence × Cu = 0.02; urine Cu: none; balance: Cu = 0.03. Pooled standard deviations: 0.15 for feces Cu, 0.01 for urine Cu, 0.17 for balance.
† Low Mg = 99 mg/2000 kcal; high Mg = 399 mg/2000 kcal.
‡ Sequence in which dietary magnesium was fed in the crossover design; mean is an average of all subjects fed diets low or high in magnesium in each copper group.
§ Number of subjects; because each sequence group started with seven subjects, *n* indicates the volunteer dropout distribution.
‖ Values are means. Caloric variation resulted in some diet Cu variation.
Cu, copper; Mg, magnesium

TABLE IV.

MAGNESIUM CONCENTRATIONS IN SERUM PLASMA AND RED BLOOD CELLS (RBC) COLLECTED THE LAST 16 D OF EACH 81-D DIETARY PERIOD*

		Low dietary Cu (1 mg/2000 kcal)							High dietary Cu (3 mg/2000 kcal)						
Diet Mg†	Sequence‡	*n*§	Serum, total (mM/L)	Plasma, ionized (mM/L)	RBC, packed (mM/L)	RBC, Hb (μM/g)	RBC, cell (μM/10^6)	RBCM, protein (μM/g)	*n*§	Serum, total (mM/L)	Plasma, ionized (mM/L)	RBC, packed (mM/L)	RBC, Hb (μM/g)	RBC, cell (μM/10^6)	RBCM, protein (μM/g)
Low	First	4	0.81‖	0.57	2.03	6.13	0.188	2.26	5	0.82	0.56	1.90	5.71	0.169	2.14
High	Second	4	0.92	0.59	2.30	6.71	0.206	2.59	5	0.88	0.57	2.00	5.86	0.176	2.41
Low	Second	5	0.84	0.55	2.17	6.46	0.189	2.55	5	0.87	0.56	2.06	5.97	0.189	2.76
High	First	5	0.85	0.57	2.12	6.50	0.189	2.43	5	0.84	0.58	1.97	5.88	0.182	2.72
Low	Mean	9	0.83	0.56	2.10	6.29	0.188	2.43	10	0.85	0.56	1.98	5.84	0.179	2.43
High	Mean	9	0.88	0.58	2.21	6.62	0.197	2.51	10	0.86	0.58	1.99	5.86	0.179	2.55

* Significant *P* values by repeated measures analysis of variance: serum total Mg: Mg × sequence × Cu = 0.003; plasma ionized Mg: Mg = 0.008; packed RBC Mg: Cu = 0.02, Mg × sequence × Cu = 0.0008; RBC (Hb) Mg: Cu = 0.009, Mg × sequence × Cu = 0.03; RBC (cell) Mg: Mg × sequence × Cu = 0.01 (Cu = 0.08); RBCM Mg: none (Mg × sequence × Cu = 0.07). Pooled standard deviations by repeated measures analysis of variance: serum total Mg = 0.03, plasma ionized Mg = 0.02, packed RBC Mg = 0.08, RBC (Hb) Mg = 0.2, RBC (cell) Mg = 0.007, RBCM Mg = 0.22.

† Low Mg = 99 mg/2000 kcal; high Mg = 399 mg/2000 kcal.

‡ Sequence in which dietary magnesium was fed in the crossover design; mean is an average of all subjects fed low magnesium or high magnesium in each copper group

§ Number of subjects; because each sequence group started with seven subjects, *n* indicates the volunteer dropout distribution.

‖ Values are means.

Cu, copper; Mg, magnesium; Hb, hemoglobin; RBC, red blood cell; RBCM, red blood cell membrane

magnesium was low than when it was high when the magnesium restriction occurred first. When the magnesium restriction occurred after 81 d of high magnesium intake, the restriction did not decrease the magnesium concentration in red blood cells on any basis; in the copper-supplemented women, the magnesium concentration actually increased when expressed on the per-cell or packed-cells basis.

Plasma copper was affected by a significant interaction between magnesium and copper intakes (Table V). When dietary magnesium was low, serum copper was lower in the women fed marginal copper than in those fed luxuriant copper. When dietary magnesium was high, low dietary copper did not significantly affect serum copper. Intracellular copper was affected differently from extracellular copper. The copper concentration in the red blood cell on a cellular, gram of hemoglobin, or packed cells basis increased during the course of the experiment, which may have been partly responsible for the significant magnesium-by-sequence effects. The dietary copper treat-

TABLE V.

COPPER CONCENTRATIONS IN PLASMA AND RED BLOOD CELLS (RBC) COLLECTED THE LAST 16 D OF EACH 81-D DIETARY PERIOD*

		Low dietary Cu (1 mg/2000 kcal)					High dietary Cu (3 mg/2000 kcal)				
Diet Mg†	Sequence‡	*n*§	Plasma, total (μM/L)	RBC, packed (μM/L)	RBC, Hb (nM/g)	RBC, cell (pM/10^6)	*n*§	Plasma, total (μM/L)	RBC, packed (μM/L)	RBC, Hb (nM/g)	RBC, cell (pM/10^6)
Low	First	4	17.3‖	10.3	31.2	0.94	5	18.3	10.7	32.4	0.94
High	Second	4	17.3	11.4	33.4	1.03	5	18.1	11.3	33.1	0.99
Low	Second	5	16.8	11.3	33.8	0.99	5	17.8	10.9	31.6	1.01
High	First	5	17.5	10.3	31.8	0.94	5	17.0	10.0	29.9	0.93
Low	Mean	9	17.1	10.8	32.4	0.97	10	17.9	10.8	32.0	0.98
High	Mean	9	17.4	10.9	32.6	0.99	10	17.5	10.7	31.5	0.96

* Significant *P* values by repeated measures analysis of variance; plasma total Cu: Cu × Mg = 0.02; packed RBC Cu: Mg × sequence × Cu = 0.002; RBC (Hb) Cu: Mg × sequence × Cu = 0.007; RBC (cell) Cu: Mg × sequence × Cu = 0.002. Pooled standard deviations: 0.5 for plasma total Cu, 0.6 for packed RBC Cu, 1.4 for RBC (Hb) Cu, 0.05 for RBC (cell) Cu.

† Low Mg = 99 mg/2000 kcal; high Mg = 399 mg/2000 kcal.

‡ Sequence in which dietary magnesium was fed in the crossover design; mean is an average of all subjects fed diets low or high in Mg in each Cu group.

§ Number of subjects; because each sequence group started with seven subjects, *n* indicates the volunteer dropout distribution.

‖ Values are means.

Cu, copper; Hb, hemoglobin; Mg, magnesium; RBC, red blood cell

TABLE VI.

COPPER ENZYMES IN BLOOD COLLECTED THE LAST 16 D OF EACH 81-D DIETARY PERIOD*

		Low dietary Cu (1 mg/2000 kcal)						High dietary Cu (3 mg/2000 kcal)					
Diet Mg†	Sequence‡	*n*§	CCO, platelet‖ ($U/10^9$ cells)	CCO, platelet (U/mg protein)	SOD, RBC# (U/Hb)	Serum Cp, ENZ (mg/L)	Serum Cp, RID (mg/L)	*n*§	CCO, platelet‖ ($U/10^9$ cells)	CCO, platelet (U/mg protein)	SOD, RBC# (U/Hb¶)	Serum Cp, ENZ (mg/L)	Serum Cp, RID (mg/L)
Low	First	4	4.54¶	1.88	3313	460	338	5	4.10	1.63	3464	478	372
High	Second	4	3.93	1.60	3553	455	331	5	3.64	1.40	3060	474	339
Low	Second	5	4.26	1.67	3004	426	303	5	3.30	1.56	3805	453	325
High	First	5	4.47	1.85	2924	471	357	5	3.65	1.53	3236	456	343
Low	Mean	9	4.40	1.77	3159	443	321	10	3.70	1.59	3634	466	349
High	Mean	9	4.20	1.72	3239	463	344	10	3.65	1.47	3148	465	341

* Significant *P* values by repeated measures analysis of variance: platelet CCO (cell): Cu = 0.03 (Mg × sequence × Cu = 0.09); platelet CCO (protein): (Cu = 0.06) (Mg × sequence × Cu = 0.06); RBC SOD: Mg = 0.03, (Mg × Cu = 0.004; serum ENZ Cp: none (Mg × sequence × Cu = 0.09); serum RID Cp: none (Mg × sequence × Cu = 0.06). Pooled standard deviations: platelet CCO (cell) = 0.52; platelet CCO (protein) = 0.20; RBC SOD = 246; ENZ Cp = 23; RID Cp = 33.

† Low Mg = 99 mg/2000 kcal; high Mg = 399 mg/2000 kcal.

‡ Sequence in which dietary magnesium was fed in the crossover design; mean is an average of all subjects fed diets low or high in Mg in each Cu group.

§ Number of subjects; because each sequence group started with seven subjects, *n* indicates the volunteer dropout distribution.

‖ Units are 1.0 μM of cytochrome C substrate oxidized per minute.

Units are amount needed to inhibit pyrogallol oxidation by 50%

¶ Values are means.

CCO, cytochrome C oxidase; Cp, ceruloplasmin; Cu, copper; ENZ, enzymatic; Hb, hemoglobin; Mg, magnesium; RID, radial immunodiffusion; SOD, copper, zinc superoxide dismutase

ments did not significantly affect red blood cell copper concentrations.

Considering the red blood cell copper findings, the results shown in Table VI were somewhat surprising. Serum ceruloplasmin levels measured enzymatically and by radial immunodiffusion were not significantly affected by the dietary treatments. The only significant effect found with cytochrome c oxidase activity was that it was higher when dietary copper was low than when it was high when expressed on the platelet basis; when expressed on a platelet protein basis, this difference only approached significance ($P = 0.06$). There also were significant red blood cell SOD changes. As shown in Table VI, magnesium deprivation elevated SOD when dietary copper was luxuriant; when dietary copper was low, magnesium deprivation did not have any effect. The interaction between dietary copper and magnesium also can be described because SOD was higher in copper-luxuriant than in copper-marginal women when dietary magnesium was low but not different between the copper groups when dietary magnesium was high.

Table VII shows that glutathione peroxidase activity, whether expressed on the lysate, cell, or gram of hemoglobin basis, was affected similarly to red blood cell copper concentration; the activity increased during the course of the experiment, which may have been partly responsible for the significant magnesium-by-sequence effects. The dietary treatments had no effect on serum glutathione concentration.

Dietary treatment affected serum lipid concentrations (Tables VIII and IX). Total cholesterol and LDL cholesterol were lower during magnesium restriction than during magnesium supplementation when the restriction occurred first. When the magnesium restriction occurred after magnesium supplementation, dietary magnesium did not affect total or LDL cholesterol in the women fed low dietary copper; in the women fed high dietary copper, the magnesium restriction resulted in an increase in these variables. High-density lipoprotein cholesterol was not significantly affected by the dietary variables. The serum concentration of Apo-A1 was significantly decreased by magnesium restriction; this effect was most marked when the magnesium restriction occurred first in the women fed low dietary copper. Serum Apo-B was affected by an interaction among copper, sequence, and magnesium. The concentration of Apo-B was significantly decreased by magnesium restriction when it occurred first in the women fed low dietary copper; dietary magnesium intake did not affect Apo-B in the other groups. There also was a tendency for Apo-B to be lower in the women fed low dietary copper.

DISCUSSION

The magnesium balance data indicated that magnesium depletion occurred when dietary magnesium was about 100 mg/2000 kcal because this intake resulted in a non-positive balance. In contrast, when dietary magnesium was high, magnesium balance was highly positive, which suggested the replenishment of magnesium stores that were low because of experimental dietary restriction or low intakes before entry into the study. The women who were fed about 400 mg of Mg/2000 kcal for 81 d first did not exhibit an average non-positive balance even after being fed only 100 mg of Mg/2000 kcal for 81 d, whereas the women who were fed the 100 mg of Mg/2000 kcal first had non-positive balance during this dietary period. This finding may have been the result of the likelihood that most women were consuming less than 400 mg of Mg/d upon admission into the study; dietary surveys indicate that it is common for postmenopausal women to consume less than the recommended amounts of magnesium.[34–37] Also, the equilibration period of 10 d at the start of the experiment, when the diet supplied 299 mg of Mg and 1 mg of Cu/2000 kcal, most likely was not of adequate length to bring the women fully into a new equilibration before beginning the study.

The magnesium balance data may have been mitigated by an unfortunate decision to make the diet high in fructose. This action was taken because animal experiments indicated that high dietary fructose is antagonistic to magnesium[38–40] and copper[41–45] metab-

TABLE VII.

GLUTATHIONE PEROXIDASE ACTIVITY IN RED BLOOD CELLS AND GLUTATHIONE IN WHOLE BLOOD COLLECTED THE LAST 16 D OF EACH 81-D DIETARY PERIOD*

		Low dietary Cu (1 mg/2000 kcal)					High dietary Cu (3 mg/2000 kcal)				
Diet Mg†	Sequence‡	*n*§	GP (U)‖	GP ($U/10^6$ RBC)#	GP (U/g Hb)#	Glutathione (mM/L)	*n*§	GP (U)‖	GP ($U/10^6$ RBC)#¶	GP (U/g Hb)#	Glutathione (mM/L)
Low	First	4	3.27¶	0.89	2.93	0.88	5	3.80	1.03	3.41	1.06
High	Second	4	3.80	1.09	3.61	0.92	5	4.39	1.19	4.37	0.95
Low	Second	5	3.68	1.01	3.53	1.06	5	3.67	1.10	3.51	0.93
High	First	5	3.50	0.97	3.35	1.02	5	3.02	0.85	2.74	0.92
Low	Mean	9	3.47	0.95	3.23	0.97	10	3.74	1.07	3.46	1.00
High	Mean	9	3.65	1.03	3.48	0.97	10	3.70	1.02	3.55	0.94

* Significant *P* values by repeated measures analysis of variance: GP (U): Mg × sequence × Cu = 0.03; GP (10^6 RBC): Mg × sequence × Cu = 0.05; GP (g Hb); Mg × sequence × Cu = 0.001; glutathione: none. Pooled standard deviations: GP (U) = 0.53; GP (10^6 RBC) = 0.19; GP (g Hb) = 0.45; glutathione = 0.06.

† Low Mg = 99 mg/2000 kcal; high Mg = 399 mg/2000 kcal.

‡ Sequence in which dietary magnesium was fed in the crossover design; mean is an average of all subjects fed diets low or high in Mg in each Cu group.

§ Number of subjects; because each sequence group started with seven subjects, *n* indicates the volunteer dropout distribution.

‖ Units are moles of glutathione oxidized per minute by 0.1 mL of RBC lysate.

Units are moles of glutathione oxidized per minute.

¶ Values are means.

Cu, copper; GP, glutathione peroxidase; Hb, hemoglobin; Mg, magnesium; RBC, red blood cell

olism and use when these elements are low in the diet. Recent findings indicate that this does not occur in humans; in fact, the opposite occurs. Milne and Nielsen found that high dietary fructose increased magnesium balance in young men with low and high dietary magnesium intakes.[46] Holbrook et al. reported a similar effect of fructose on magnesium balance.[47]

Holbrook et al.[47] and Milne and Nielsen[48] also found that feeding fructose instead of starch had a positive rather than a negative effect on copper balance in young men. Thus, the high dietary fructose in the present experiment also probably had a mitigating effect on the copper balance data, especially when dietary copper was low. Nonetheless, the copper balance data indicate that the women fed low dietary copper had a different copper status than those fed high dietary copper. Those women fed low dietary copper throughout the study probably had a marginal copper status, whereas those fed 3 mg of Cu/d had an optimal copper status.

Although the balance data showed that magnesium intake did not significantly affect copper retention and that copper intake did not markedly affect magnesium retention, some blood variables indicated that copper intake affected the distribution of magnesium in the body. The concentrations of serum and red blood cell

TABLE VIII.

TC, LDL-C, AND HDL-C IN SERUM COLLECTED THE LAST 16 D OF EACH 81-D DIETARY PERIOD*

		Low dietary Cu (1 mg/2000 kcal)				High dietary Cu (3 mg/2000 kcal)			
Diet Mg†	Sequence‡	*n*§	TC (mM/L)	LDL-C (mM/L)	HDL-C (mM/L)	*n*§	TC (mM/L)	LDL-C (mM/L)	HDL-C (mM/L)
Low	First	4	5.79‖	3.90	1.18	5	5.22	3.08	1.09
High	Second	4	6.41	4.42	1.22	5	5.72	3.41	1.10
Low	Second	5	4.99	3.00	1.25	5	5.94	4.09	1.37
High	First	5	4.94	2.95	1.33	5	5.46	3.65	1.33
Low	Mean	9	5.38	3.47	1.22	10	5.59	3.59	1.23
High	Mean	9	5.69	3.70	1.27	10	5.59	3.54	1.22

* Significant *P* values by repeated measures analysis of variance: TC: Mg × sequence × Cu = 0.0003; LDL-C: Mg × sequence × Cu = 0.001; HDL-C: none. Pooled standard deviations: TC = 0.24; LDL-C = 0.22: HDL-C = 0.09.

† Low Mg = 99 mg/2000 kcal; high Mg = 399 mg/2000 kcal.

‡ Sequence in which magnesium was fed in the crossover design; mean is an average of all subjects fed diets low or high in Mg in each Cu group

§ Number of subjects; because each sequence group started with seven subjects, *n* indicates volunteer dropout distribution.

‖ Values are means

Cu, copper; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Mg, magnesium; TC, total cholesterol

TABLE IX.

APOLIPOPROTEINS A1 AND B AND TRIACYLGLYCEROL CONCENTRATIONS IN SERUM COLLECTED THE LAST 16 D OF EACH 81-D DIETARY PERIOD*

		Low dietary Cu (1 mg/2000 kcal)				High dietary Cu (3 mg/2000 kcal)			
Diet Mg†	Sequence‡	*n*§	ApoA1 (g/L)	ApoB (g/L)	TG‖	*n*§	ApoA1 (g/L)	ApoB (g/L)	TG‖
Low	First	4	1.37#	1.08	130	5	1.54	1.29	181
High	Second	4	1.68	1.39	147	5	1.51	1.25	202
Low	Second	5	1.64	1.03	122	5	1.65	1.28	96
High	First	5	1.72	1.02	112	5	1.73	1.21	93
Low	Mean	9	1.50	1.05	126	10	1.59	1.29	132
High	Mean	9	1.70	1.21	129	10	1.62	1.23	137

* Significant *P* values by repeated measures analysis of variance: ApoA1: Mg = 0.05; ApoB: Mg × sequence × Cu = 0.05; TG: Mg × sequence × Cu = 0.004. Pooled standard deviations: ApoA1 = 0.15; ApoB = 0.12.

† Low Mg = 99 mg/2000 kcal; high Mg = 399 mg/2000 kcal.

‡ Sequence in which magnesium was fed in the crossover design; mean is an average of all subjects fed diets low or high in Mg in each Cu group

§ Number of subjects; because each sequence group started with seven subjects, *n* indicates volunteer dropout distribution.

‖ Data was log transformed for statistical analysis

Values are means

ApoA1, apolipoprotein A1: ApoB, apolipoprotein B; Cu, copper; Mg, magnesium; TG, triacylglycerol

magnesium were decreased by magnesium restriction regardless of dietary copper when it occurred first. In contrast, when magnesium restriction followed magnesium supplementation, the restriction seemed to increase these variables when dietary copper was high and resulted in no change in the variables when dietary copper was low.

Based on the blood magnesium variables and magnesium balance data, the magnesium status of the women at the end of the dietary period when the low magnesium diet was fed first most likely was lower than that of the women at the end of the dietary period when the low magnesium diet was fed second (the high magnesium fed first for 81 d). The women in the latter group probably were able to maintain close to an adequate magnesium status by using magnesium stores built during the high magnesium dietary period. Thus, when one examined other data from this experiment, the likelihood that there were three different magnesium states, mildly deficient, marginally adequate or inadequate, and luxuriantly adequate, needs to be considered.

Plasma copper concentration and platelet cytochrome c oxidase findings supported the copper balance data in indicating the women fed low dietary copper throughout the study had a marginal copper status. The plasma copper concentration was lower when dietary copper was 1 mg/2000 kcal than when it was 3 mg/2000 kcal. An initial impression was that the cytochrome c oxidase activity change was in the wrong direction to indicate copper depletion when the dietary copper was low because severe copper deficiency in animals results in a decrease in the activity.[49] However, other human experiments in which copper status was marginal (not markedly deficient), platelet cytochrome c oxidase tended to be higher than when the copper intake was luxuriant and when other nutrients that could affect this enzyme were varied (sulfur amino acids and zinc)[50,51]; these subjects also showed changes in plasma copper similar to that seen in the present experiment. Although the marginal copper status apparently affected oxidative metabolism, copper status definitely was no more than marginally deficient because the low intake did not significantly affect serum ceruloplasmin concentrations measured enzymatically and by radial immunodiffusion.

As cytochrome c oxidase activity was increasing in the platelets, glutathione peroxidase activity in platelets was decreasing, or vice-versa. The meaning of this change is unclear, but it indicated that oxidative metabolism was affected in the experiment. The changes may have been partly caused by the copper and magnesium treatments because copper and magnesium deficiencies cause detrimental changes in oxidative metabolism including decreased glutathione peroxidase activity in animals[49,52–54] and perhaps humans.[55] In the present experiment, the magnesium and copper restrictions did not cause a significant change in serum glutathione. Perhaps the dietary copper and magnesium treatments negated each other so that no glutathione effect was seen because copper deprivation increases circulating and hepatic glutathione in rats,[56,57] and magnesium deprivation decreases glutathione concentrations in red blood cells of rats,[54] cultured cortical neurons,[58] and perhaps red blood cells of humans.[59]

Perhaps the most convincing evidence of a relation between dietary copper and magnesium that affects oxidative metabolism is the SOD data. Magnesium deficiency increased red blood cell SOD, but the increase depended on copper being adequate in the diet. This finding suggested that an increased oxidant stress caused by magnesium deprivation induces an increased need for SOD activity, which cannot be adequately met when copper status is low. It also suggested that a combined magnesium and copper deficiency most likely would lead more quickly to detrimental consequences than would either alone.

In other human experiments at the Grand Forks Human Nutrition Research Center, magnesium deprivation resulted in decreased serum total and LDL cholesterol.[60,61] A significant magnesium effect on these variables was not found in the present study. Although serum total and LDL cholesterol were decreased during magnesium restriction when it occurred first, when it occurred second, there was no decrease. Possibly, the high fructose content of the diet had an effect on the cholesterol findings. High dietary fructose has been shown to increase serum cholesterol.[62–64] Thus, the high fructose diet over the duration of the experiment may have negated any lowering effect of magnesium deprivation when it occurred in the last 81 d of the experiment. Although the finding that a combined magnesium deficiency with a low intake of copper has a detrimental effect on lipid metabolism was not clearly shown by the cholesterol data, the Apo-A1 data suggested that it does. Magnesium restriction overall decreased Apo-A1, but the effect was most marked when magnesium restriction was first in women fed low dietary copper. Decreased Apo-A1 might be

considered an undesirable change because it is associated with high-density lipoprotein cholesterol metabolism.

The present findings indicate that, although substantial homeostatic mechanisms work to maintain an adequate magnesium status when intake is low, a dietary intake of about 100 mg/2000 kcal eventually leads to depleted body stores and changes in oxidative and lipid metabolism. However, in short-term depletion experiments, the response to magnesium restriction can be influenced by other dietary factors including copper intake and perhaps prior magnesium intake and dietary carbohydrate form, especially fructose. In other words, the present findings suggest that, if subjects are allowed to consume luxuriant amounts of magnesium for a substantial period (weeks) before magnesium depletion is initiated, inducing a magnesium deficiency in the subjects may take an extended period even when intakes are quite low, and that low dietary copper may enhance some of the oxidative and lipid metabolism changes induced by magnesium deficiency when it is achieved. The findings in this experiment also indicate that it is possible to induce a magnesium deficiency in postmenopausal women by restricting the amount of magnesium in the diet, and contrary to findings in short-term balance studies,[65,66] intakes similar to the low intakes in the present study clearly are not adequate for postmenopausal women.

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